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ORIGINAL ARTICLE

A New Species of the Genus *Rhacophorus* (Anura: Rhacophoridae) from Southern China

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Abstract A new species of rhacophorid of the genus *Rhacophorus* is described from the Shiwandashan National Nature Reserve, Guangxi, Southern China. *Rhacophorus pinglongensis* **sp. nov.** is compared with congeners from China and other parts of Southeast Asia. The new species is distinguished from its congeners by combination of following characters: (1) small size (adult male, SVL 32.0-38.5 mm); (2) smooth and green dorsum; (3) flanks, axilla, ventral surface of forearms, inguinal, anterior and posterior surfaces of thighs, ventral surface of shank, and dorsal surface of feet covered with black blotches with white spots or white spots with a faint orange tint; (4) ventral surface of feet and webbing tangerine; (5) supratympanic fold weak; (6) outer margin of forearms and feet with low dermal ridges; (7) calcars absent on heels; (8) iris silver, diffusing to ecru laterally with light khaki ring along margin. The new species is closely related to *R. dorsoviridis*, *R. moltrechti*, and *R. nigropunctatus* based on adult morphology. Currently, this species is known only from mid-elevation montane evergreen forest in the Shiwandashan National Nature Reserve, Guangxi, China.

Keywords Tree frog, *Rhacophorus pinglongensis* **sp. nov.**, Rhacophoridae, Southern China

1. Introduction

Rhacophoridae Hoffman, 1932 with approximately 393 species of frogs is one of the most diverse anuran families (Frost, 2015). The rhacophorid tree frogs are widely distributed across Japan, China, India, Indochina, Sundaland to Sulawesi and the Philippines (Streicher *et al.*, 2014; Hamidy and Kurniati, 2015; Frost, 2015). Among rhacophorid tree frogs, the genus *Rhacophorus* Kuhl and Van Hasselt, 1822 currently contains approximately 88 species (Frost, 2015). Fei *et al.* (2012) suggested that twenty-seven species of *Rhacophorus* occur in China. In the last decade, some new tree frogs are continuously reported in China (Zhao *et al.*, 2005; Rao *et al.*, 2006; Mo *et al.*, 2008; Li *et al.*, 2012a).

Guangxi, locating at Southern China, has the particular geographic location and complicated environment and contains a rich diversity of amphibian. Mt. Shiwandashan, lying in the south of Guangxi, covers 2 600km^2, most of which are natural forest. During field works in the Shiwandashan National Nature Reserve, Guangxi, Southern China (Figure 1 A), from 2009 to 2012, we discovered a small species of tree frog, which are closely related to *Rhacophorus dorsoviridis* Bourret, 1937, *R. moltrechti* Boulenger, 1908 and *R. nigropunctatus* Liu, Hu, and Yang, 1962 based on morphology, but that differs morphologically from Chinese and all Southeast Asian members of *Rhacophorus*. Herein, we describe these tree frogs as new.

2. Material and Methods

Specimens were deposited at the Natural History Museum of Guangxi. Morphological data were recorded from specimens fixed in 8% formalin and then stored in 70% ethanol. The following morphometric data were taken with digital calipers (to the nearest 0.1 mm): SVL (snout-vent length); HL (head length from tip of snout to rear of jaws); HW (head width at the commissure of the jaws); SNT (snout length from tip of snout to the anterior corner of eye); ED (diameter of the exposed portion of the eyeball); IOD (interorbital distance); TD (horizontal diameter of tympanum); UEW (upper eyelid width,

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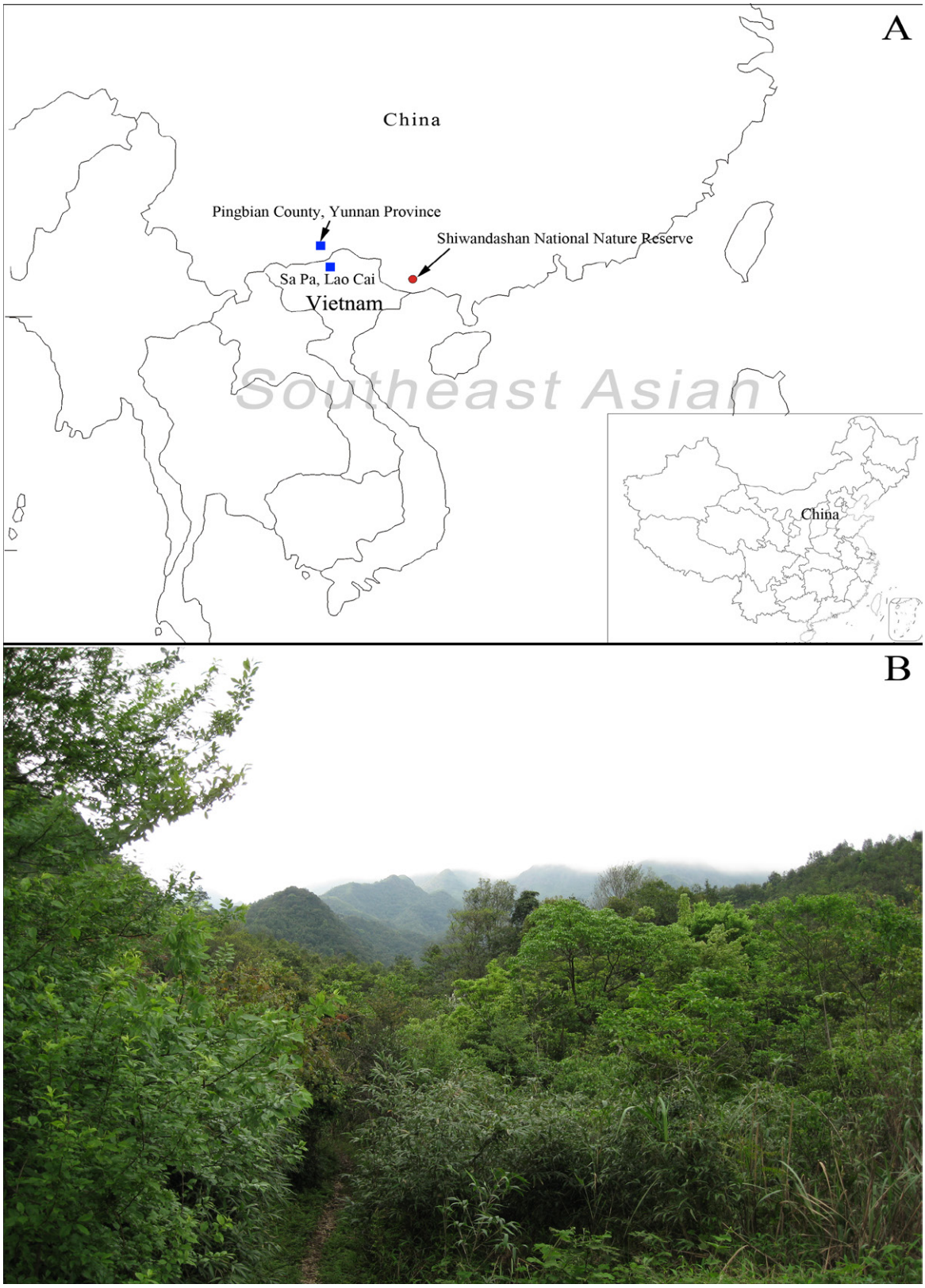

Figure 1 (A) Localities of *Rhacophorus pinglongensis* **sp. nov.** (solid circle) and *R. dorsoviridis* (solid square); (B) Habitat of *Rhacophorus pinglongensis* **sp. nov.**

greatest width of upper eyelids); TED (distance from anterior edge of tympanum to posterior corner of the eye); IN (internarial space); EN (distance from front of eye to nostril); TIB (tibia length with the hindlimb flexed); FLL (length of forelimb from tip of disk of finger III to axilla); FTD (maximal diameter of disc of third finger); HLL (length of hindlimb from tip of disk of fourth toe to groin); THL (thigh length, from vent to knee); HTD4 (diameter of fourth toe tip, greatest diameter of disc on fourth toe). The webbing formula is given as proposed by Myers and Duellman (1982). Museum acronyms: NHMG for the Natural History Museum of Guangxi, Nanning, Guangxi Province, China; CIB for the Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, China.

Advertisement calls of three males were recorded in the field using ICD recorder (Sony ICD–TX50). Calls were recorded at a distance of about 0.1-0.3 m, and ambient temperature was recorded using TP–2200 (A–volt). Call recordings were analyzed using Raven Pro 1.3 beta version (http://www.birds.cornell.edu/raven) with default settings.

To display genetic variation and preliminarily determine the generic placement of the new species, we sequenced this species and compared with other rhacophorid tree frogs in the genus *Rhacophorus* obtained from GenBank. Total genomic DNA was extracted from muscle tissues stored in 100% ethanol, using DNeasy tissue extraction kits (QIAGEN). The primers employed in this study followed Wilkinson *et al.* (2002) and amplified 12S and 16S fragments including the complete t-RNA^{valine}. We used Bio-Rad S1000 (USA) to amplify genes following the standard PCR protocols (5 min at 94°C, then 35 cycles at 94°C for 50s, 55°C for 50s, and 72°C for 50s, with a final 10 min extension at 72°C). PCR products were purified using QIAquick Gel Extraction Kit (QIAGEN) according to the manufacturer's protocols and directly sequenced with an ABI 3730 automated DNA sequencer. New mitochondrial DNA fragments were submitted for BLAST search in GenBank to ensure that the required sequences had been amplified (Altschul *et al.*, 1997). New sequences were deposited in GenBank under the accession numbers KU170683-4. *P*-distance pairwise sequences divergence between species using a ~530 bp mtDNA 16S fragment was calculated using MEGA version 5 (Tamura *et al.*, 2011). Missing data and indels were removed using Gblocks (Castresana, 2000) with default settings. After removing poorly aligned regions, a 1607 bp matrix was used to reconstruct phylogenetic relationships, using maximum likelihood (ML) and Bayesian inference (BI). BI was implemented in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Details for BI were given in Li *et al.* (2012b). ML analyses were performed on the RAxML Web server (http://phylobench.vital-it.ch/raxml-bb/) with 100 rapid bootstrap replicates (Stamatakis *et al.*, 2008). Comparative mtDNA sequences examined in this study were listed in Appendix.

3. Results

***Rhacophorus pinglongensis* sp. nov.**

Holotype NHMG200903002, adult male, from the Shiwandashan National Nature Reserve, Guangxi, China (22.457º N, 107.043º E; alt. 530 m). Collected by Yunming Mo and Shichu Zhou on 23 March 2009 (Figure 2 A, B).

Paratypes NHMG200903001, NHMG200903003-004, adult male, from the same site as the holotype, collected by Yunming Mo and Shichu Zhou on 23 March 2009; NHMG201002003-008, NHMG201002010, 201002011, 201002013, adult males, from the same site as the

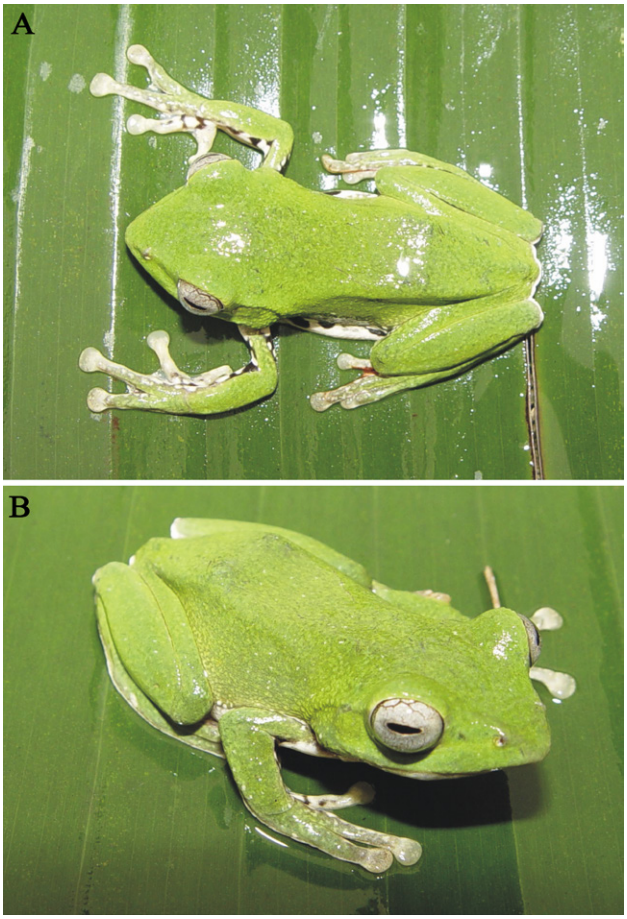

Figure 2 Dorsal(A), and lateral (B) views of the holotype NHMG200903002.

holotype, collected by Yunming Mo, Wei Zhang, and Shichu Zhou on 25 February 2010.

Diagnosis The new species is assigned to the genus *Rhacophorus* by Y-shaped distal end of terminal phalanx, the presence of intercalary cartilage between the terminal and penultimate phalanges of digits, webbed fingers, tips of digits expanded into large disks with circummarginal grooves, vomerine teeth, a supracloacal dermal ridge, eyes large and horizontal pupil (Liem, 1970; Duellman and Trueb, 1986; Brown and Alcala, 1994; Wilkinson and Drewes, 2000; Rowley *et al.*, 2010). The new species distinguishes from its congeners by the combination of the following characters: (1) small size (adult male, SVL 32.0–38.5 mm, *n*=13; females are unknown); (2) smooth and green dorsum; (3) flanks, axilla, ventral surface of forearms, inguinal, anterior and posterior surfaces of thighs, ventral surface of shank, and dorsal surface of foot covered with black blotches with white spots or white spots with a faint orange tint; (4) ventral surface of feet and webbing tangerine; (5) supratympanic fold weak; (6) outer margin of forearms and feet with low dermal ridges; (7) calcars absent on heels; (8) iris silver, diffusing to ecru laterally with light khaki ring along margin (Figure 2 and Figure 3A, B).

Description of holotype Body moderately robust, SVL 38.2 mm; head width nearly equal to length; snout rounded in lateral profile, protruding; canthus rostralis indistinct, loreal region sloping; interorbital region slightly convex; nostril oval, laterally positioned, slightly protuberant, nearer tip of snout than eye; internarial distance greater than distance from anterior margin of eye to nostril (ID/EN=1.26); pupil horizontal; eye diameter greater than eye to nostril distance (ED/EN=1.21); pineal ocellus invisible; interorbital width greater than upper eyelid width (IOD/UEW=1.28); tympanum barely visible externally, tympanic rim slightly elevated relative to skin of temporal region, diameter less than eye diameter (TD/ED=0.64); weak supratympanic fold extending to axilla; vomerine ridge present, in two oblique groups, closer to choanae than to each other, separated by a distance about as long as each groups; choanae oval; tongue attached anteriorly, deeply notched posteriorly; external single subgular vocal sac (Figure 2).

Forelimbs relatively robust, relative length of fingers I<II<IV<III; tips of all except first finger with well-developed disks with horizontal circummarginal grooves, disks relatively wide compared to finger width, third finger disk width less than tympanum diameter (0.72 mm); fingers moderately webbed, webbing formula: I 1^{-}–1^{-} II 1^{+}–1^{+} III 2^{-}–2^{-} IV; subarticular tubercles prominent, rounded, formula 1, 1, 2, 2; out palmar tubercle absent (Figure 3C); thenar tubercle absent; prepollex tubercle indistinct; nuptial pads present. Relative length of toes I<II<III<V<IV; tips of toes disks with distinct circummarginal grooves; disks slightly less than those on fingers; toes moderately webbed, webbing formula: I 1^{+}–1^{-} II 2^{+}–2^{-} III 2^{+}–3^{-} IV 3^{-}–2^{+} V; subarticular tubercles rounded, distal subarticular tubercles distinct, formula 1, 1, 2, 3, 2; inner metatarsal tubercle oval, elongated; outer metatarsal tubercle and additional supernumerary tubercle absent (Figure 3D). Dorsal skin smooth; ventral surface of chest, venter, and thighs coarsely granular; dorsal surface of limbs smooth; ventral surface of forearms smooth; cloaca and posterior surface of thighs granular; tarsal fold present; outer margin of forearms and feet with low dermal ridges.

Color of holotype in life Dorsal surface green; flanks, axilla, ventral surface of forearms, inguinal, anterior and posterior surfaces of thighs, ventral surface of shanks, dorsal surface of feet, and fingers I, II, III covered with black blotches with white spots or white spots with a faint orange tint; ventral surface of feet and webbing tangerine; venter and chest cream; throat white with slightly gray background; groin region and ventral surfaces of thighs cream; iris silver, diffusing to ecru laterally with light khaki ring along margin, pupil horizontal, black (Figure 2 and Figure 3).

Color of holotype in preservative Dorsum dark brown; tangerine of all part of body and limbs faded to cream; color of the iris faded to bluish-gray; color of the throat faded to light gray.

Measurements of holotype (in mm) SVL 38.2, HL 15.2, HW 15.3, SNT 6.9, ED 4.7, IOD 5.5, TD 2.9, UEW 4.3, TED 1.4, IN 4.9, EN 3.9, TIB 17.0, FLL 21.6, FTD 2.1, HLL 55.0, THL 17.1, HTD4 1.8.

Male secondary sexual characters Nuptial pad present; external single subgular vocal sac; linea musculina present beneath belly.

Advertisement call In the field, fifteen advertisement calls from three type specimens were recorded. Ambient air temperature was 18.4 °C. Advertisement calls consisted of six short clicks given at irregular intervals, often with long pauses between bouts of clicking (range 2.5–101.4 s, *n*=15). The dominant frequency spectrum of the notes lay between 1.6 and 3.0 kHz. Clicks have prominent harmonics at 1.6–3.0 kHz (Figure 4).

Molecular analyses Genetic divergence between *R. pinglongensis* **sp. nov.** and all homologous sequences available in *Rhacophorus* with green dorsum were calculated with *P*-distance. The results range from 2.2%–

12.2 % (Table 1). Among them, *R. pinglongensis* **sp. nov.** and *R. dorsoviridis* (JX219427) (Accession no. of GenBank) have the smallest genetic divergence, at 2.2%, but the genetic divergence between *pinglongensis* **sp. nov.** and *R. dorsoviridis* (JX219423) reaches 5.4%. ML and BI trees have a similar topology and are consistent with Li *et al.*, (2012b). Based on the 1607 bp matrix, ML and BI trees indicated that *R. pinglongensis* **sp. nov.** was closely related to *R. dorsoviridis* (JX219427) with robust values (BP=100; BBP=1.00) (Figure 5).

Variation Measurements of thirteen specimens are shown in Table 2. In life, the coloration of the paratypes was similar to that of the holotype. Irregularly black blotches on flanks are discrete in NHMG200903001, NHMG200903002, NHMG201002006, NHMG201002008, and NHMG201002013. In preservative, NHMG200903001, NHMG200903002, NHMG200903004, NHMG201002003, NHMG201002008, and NHMG201002010 have a dark dorsum, the other specimens have a brown dorsum. NHMG201002004, NHMG201002005, NHMG201002007, NHMG201002010, NHMG201002011, NHMG201002013 have a white pupil in preservative, the rest has a gray pupil. All tangerine is faded in preservative.

Etymology This species is named after the type locality, Mt. Pinglong. The suggested English name is the Pinglong tree frog.

Ecology The holotype and paratypes were found in montane evergreen forest. Females and larval stages of *R. pinglongensis* **sp. nov.** remain unknown. Sympatric species include *Amolops ricketti* Boulenger, 1899, *Microhyla pulchra* Hallowell, 1861, *M. fissipes* Boulenger, 1884, *M. heymonsi* Vogt, 1911, *Odorrana schmackeri* Boettger, 1892, *Polypedates megacephalus* Hallowell, 1861, *P. mutus* Smith, 1940, *Rhacophorus dennysi* Blanford, 1881, *Sylvirana guentheri* Boulenger, 1882 and *S. maosonensis* Bourret, 1937.

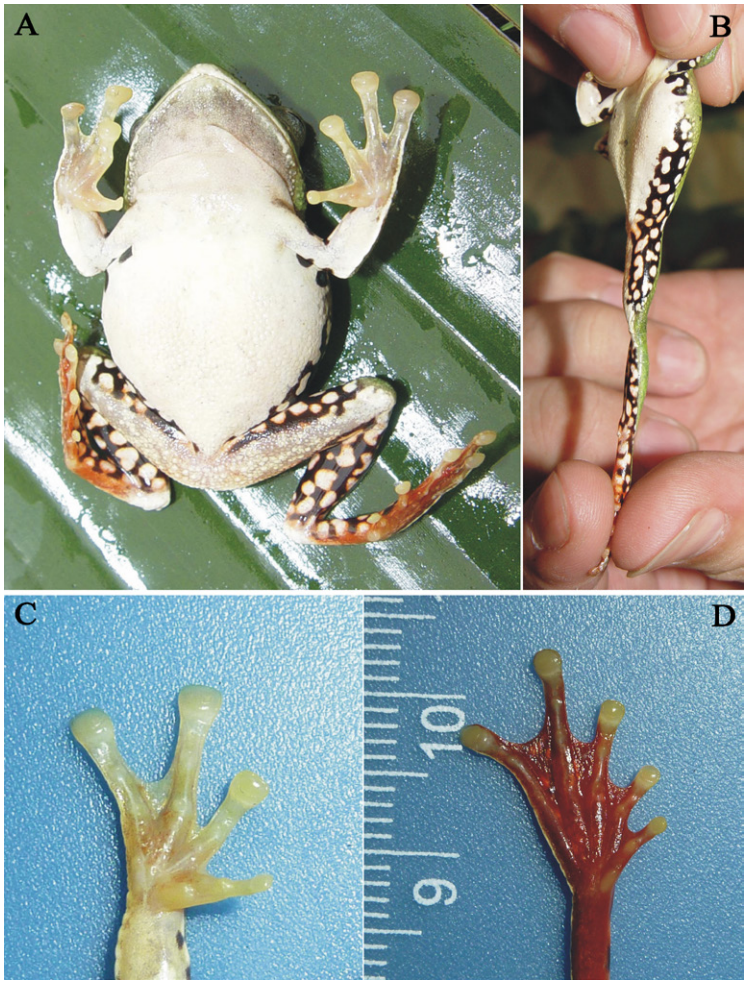

Figure 3 Ventral (A) and lateral (B) view of the holotype NHMG200903002, ventral surface of right hand (C) and right foot (D) of the holotype NHMG200903002.

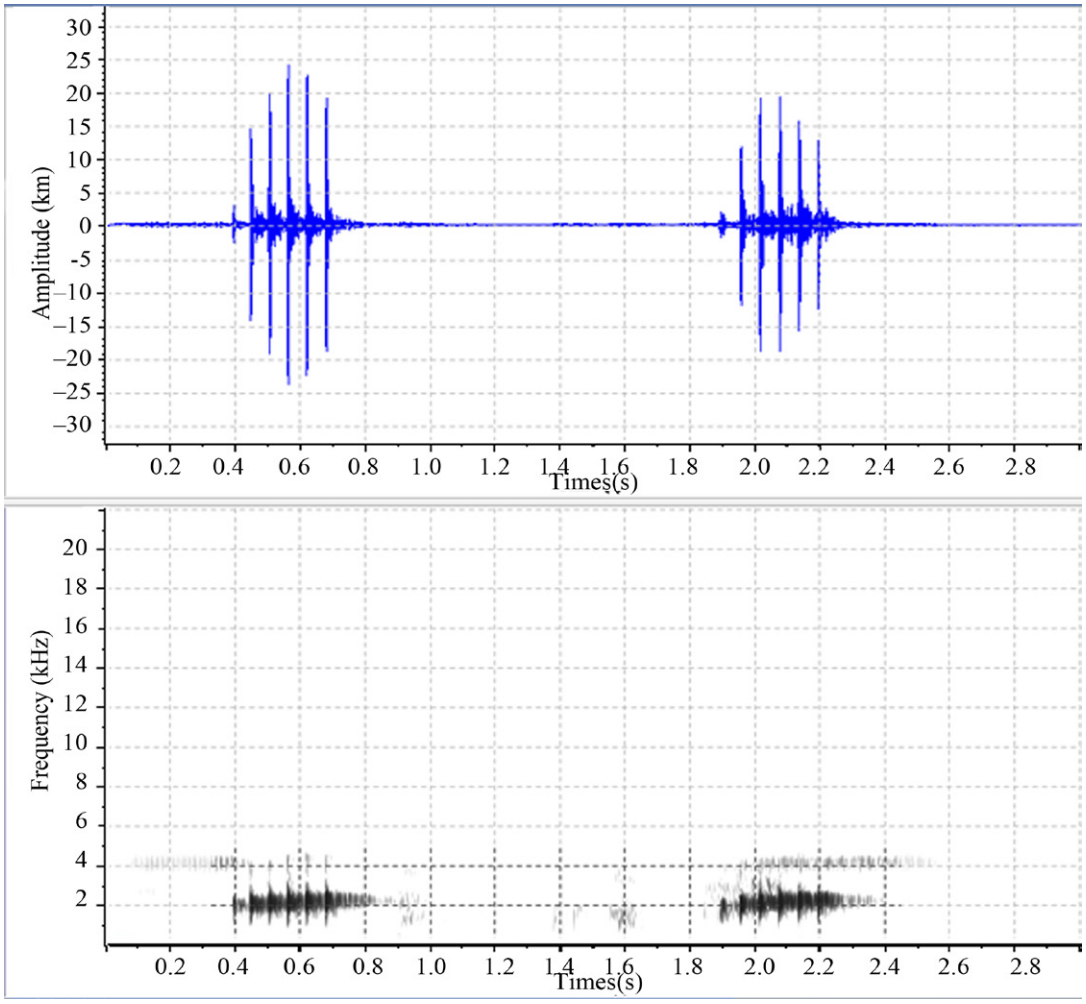

Figure 4 Oscillogram (top) and corresponding audiospectrogram (bottom) of the two-note advertisement call of a male *Rhacophorus pinglongensis* **sp. nov.** (NHMG201003003). Ambient air temperature was 18.4 °C.

Table 1 Uncorrected pairwise distances (*P*-distance) (in %) with green dorsum tree frogs in *Rhacophorus* from GenBank available based on mitochondrial 16S rRNA sequences (~530 bp).

ID	Species	1	2	3	4	5	6	7	8	9	10	11	12
1	*R. burmanus* (JX219422)												
2	*R. chenfui* (EF564537)	5.0											
3	*R. dennysi* (DQ019609)	6.4	7.8										
4	*R. dorsoviridis* (JX219423)	4.4	6.2	7.0									
5	*R. dorsoviridis* (JX219427)	4.6	5.6	7.8	5.4								
6	*R. feae* (EF564546)	4.0	4.6	6.0	4.6	4.8							
7	*R. hongchibaensis* (JN688882)	4.0	6.4	6.6	4.4	5.4	4.4						
8	*R. kio* (JQ288096)	10.6	11.1	11.2	11.6	12.1	11.0	10.6					
9	*R. maximus* (HM448032)	5.8	7.0	6.8	5.4	6.8	4.6	5.8	11.1				
10	*R. minimus* (EF564560)	4.2	6.6	6.4	5.0	4.8	5.0	1.8	10.8	5.2			
11	*R. moltrechti* (DQ468676)	3.8	5.2	6.6	4.6	5.0	4.4	3.8	10.1	5.4	4.0		
12	*R. nigropunctatus* (JX219425)	4.2	5.8	6.8	0.6	5.2	4.2	4.6	11.4	5.0	5.2	4.4	
13	*R. pinglongensis* **sp. nov.**	4.4	5.8	8.0	5.4	2.2	4.6	5.6	12.2	6.8	5.0	5.4	5.2

Table 2 Measurements (in mm) of *Rhacophorus pinglongensis* **sp. nov.**. Abbreviations defined in the text.

Character (mm)	Male (*n*=13)	
	Range	Mean ± S.D.
SVL	32.0–38.5	35.9 ± 2.3
HL	12.3–15.2	13.8 ± 1.1
HW	12.7–15.7	14.5 ± 1.1
SNT	5.5–7.2	6.3 ± 0.6
ED	4.3–5.5	4.8 ± 0.3
IOD	4.4–5.5	5.0 ± 0.4
TD	2.2–3.1	2.6 ± 0.3
UEW	3.3–4.7	3.9 ± 0.4
TED	0.8–1.6	1.1 ± 0.3
IN	4.1–5.3	4.5 ± 0.3
EN	2.6–3.9	3.2 ± 0.4
TIB	15.3–18.1	16.3 ± 0.9
FLL	17.3–23.6	19.7 ± 1.8
FTD	1.7–2.3	2.1 ± 0.2
HLL	42.7–57.4	48.8 ± 5.2
FL	15.6–18.3	17.0 ± 0.9
HTD4	1.5–2.0	1.8 ± 0.1

Comparison Smooth and green dorsum, flanks, axilla, ventral surface of forelimbs, inguinal, anterior and posterior surfaces of thighs, ventral surface of shank, and dorsal surface of foot covered with black blotches with white spots or white spots with a faint orange tint, ventral surface of foot and webbing tangerine, supratympanic fold weak, and outer margin of forearm and foot with low dermal ridges distinguish the new species from all Southeast Asian congeners.

The following tree frogs that have brown, grayish brown, reddish brown, or orange red background color on dorsum can be distinguished from this new species (additional distinguishing characters in parentheses): *R. annamensis* Smith, 1924 (fingers broadly webbed, the membrane between the outer three fingers reaching the discs; toes fully webbed, their discs considerably smaller than those of the fingers; a fold from the eye to the shoulder; dark cross-bars upon the limbs) (Smith, 1924); *R. calcaneus* Smith, 1924 (outer two fingers 3/4 webbed, inner two 1/4 webbed; toes nearly fully webbed; a strong fold from the eye to the shoulder; heel with a well-marked pointed terminal tubercle; a yellow line extending from the tip of the nose along the canthus rostralis and along the outer edges of the upper and lower eyelids (Smith, 1924); *R. exechopygus* Inger, Orlov, and Darevsky, 1999 (outer fingers fully webbed, forearm and tarsus with crenulated dermal ridge) (Inger *et al.*, 1999); *R. cyanopunctatus* Manthey and Steiof, 1998 (throat and chest white with brown speckles; snout short and angular with a well-defined ridge running from the eye to the snout; dorsum light brown with medium brown patches) (Manthey and Steiof, 1998); *R. hoanglienensis* Orlov, Lathrop, Murphy and Ho, 2001 (a pointed projection on heels; irregular black and brown spots on dorsum; loreal region dark brown) (Orlov *et al.*, 2001; Bain and Truong, 2004); *R. jarujini* Matsui and Panha, 2006 (a narrow darker brown bar crossing upper eyelids; back with irregular, darker brown crossbands) (Matsui and Panha, 2006); *R. laoshan* Mo, Jiang, Xie and Ohler, 2008 (dark brown stripe on limbs) (Mo *et al.*, 2008); *R. marmoridorsum* Orlov, 2008 (dorsum flesh colored with marble chocolate) (Orlov *et al.*, 2008); *R. orlovi* Ziegler and Köhler, 2001 (dark brown bar between eyes, loreal region and supratympanic fold dark brown with irregular yellow patches) (Ziegler and Köhler, 2001); *R. rhodopus* Liu and Hu, 1959 (temporal fold distinct; small rounded tubercles all over the thorax; reddish brown on the head, back and dorsal sides of the limbs; with dark brown mark on the back of the shoulder region and with dark brown transverse bars on the posterior dorsum of the body, some small black spots scattered on the whole back; males with a single internal subgular vocal sac) (Liu and Hu, 1959); *R. spelaeus* Orlov, Gnophanxay, Phimminith and Phomphoumy, 2010 (dorsum grayish brown with dark irregular spots; venter light gray with dark specks) (Orlov *et al.*, 2010); *R. translineatus* Wu, 1977 (snout with a conspicuous conical dermal protuberance; a triangular skin flap at the outer tibiotarsal region; more than then transverse dark brown bands on the dorsum) (referring to Sichuan Institute of Biology Herpetology Department, 1977); *R. tuberculatus* Anderson, 1871 (fingers broadly webbed, the membrane reaching the disks of the second and fourth fingers; a strong fold from the eye over the tympanum to the shoulder; under surface of thighs granular, with scattered large round tubercles; brownish yellow below with a blackish region round the vent, a short way along the thighs; the tubercles of abdomen and thighs are darker (Anderson, 1871); *R. vampyrus* Rowley, Le, Thi, Stuart and Hoang, 2010 (pale tan to brick red dorsum; flanks and anterior and posterior surface of thighs black; gray to black webbing between fingers and toes) (Rowley *et al.*, 2010); *R. verrucopus* Huang, 1983 (a pointed projection on heels; forearm and tarsus with tuberculous crenulated dermal ridge) (Huang, 1983).

Rhacophorus burmanus Andersson, 1939, *R. dennysi* Blanford, 1881, *R. duboisi* Ohler, Marquis, Swan and

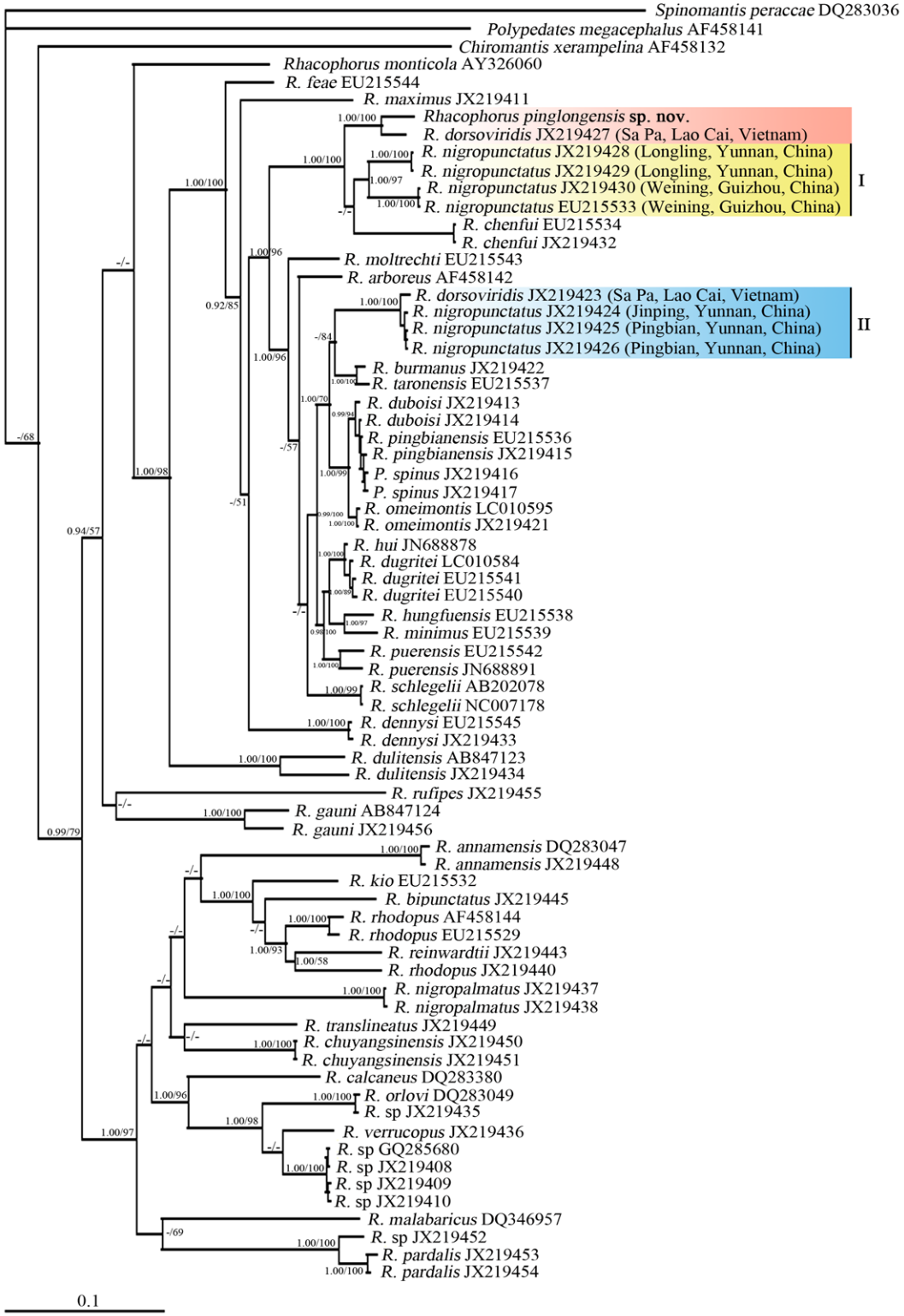

Figure 5 Bayesian inference tree reconstructed from 12S rRNA, $tRNA^{val}$ and 16S rRNA mitochondrial genes with *Spinomantis peraccae* and *Chiromantis xerampelina* as outgroups. Numbers above branches represent bootstrap supports for Bayesian posterior probabilities (BPP) (>0.9 retained)/bootstrap support for maximum likelihood analyses (>50 retained); '–' represents BPP and bootstrap proportions lower than 90% and 50%, respectively.

Grosjean, 2000, *R. feae* Boulenger, 1893, *R. kio* Ohler and Delorme, 2006, *R. maximus* Günther, 1858, *R. omeimontis* Stejneger, 1924, *R. prasinatus* Mou, Risch and Lue, 1983, and *R. reinwardtii* Schlegel, 1840 have green ground color on dorsum, but the SVL of these species is greater than 50 mm (Ohler *et al.*, 2000; Ohler and Delorme, 2006; Ohler, 2009; Fei *et al.*, 2012). *Rhacophorus duboisi*, *R. dugritei*, *R. hongchibaenis* Li, Liu, Chen, Wu, Murphy, Zhao, Wang and Zhang, 2012, *R. omeimontis*, *R. pingbianensis* Kou, Hu and Gao, 2001, and *R. zhaojuensis* Wu and Zheng, 1994 have black or brown spots or blotches on dorsum (Wu and Zheng, 1994; Ohler *et al.*, 2000; Kou *et al.*, 2000; Fei *et al.*, 2012; Li *et al.*, 2012a). *Rhacophorus arvalis* Lue, Lai and Chen, 1995 lacks black blotches on flanks and anterior and posterior surfaces of thighs, and has a white line on flanks (Lue *et al.*, 1995); *R. aurantiventris* Lue, Lai and Chen, 1994 and *R.chenfui* Liu, 1945 lack spots on anterior and posterior surfaces of thighs; *R. hungfuensis* Liu and Hu, 1961 lacks spots on flanks and thighs; *R. leucofasciatus* Liu and Hu, 1962 has a white line running from tip of snout to flanks; *R. minimus* Rao, Wilkinson and Liu, 2006 has a white line from tip of snout along the upper lip and shoulder to insertion of hind limbs (Rao *et al.*, 2006); *R. prasinatus* Mou, Risch and Lue, 1983 has a white line extending from tip of snout to flanks, with black spots beneath the line (Fei *et al.*, 2012); *R. taipeianus* Liang and Wang, 1978 has a yellowish white abdomen and several dark brown spots on inner thighs (Liang and Wang, 1978); *R. wui* Li, Liu, Chen, Wu, Murphy, Zhao, Wang and Zhang, 2012 has irregularly distributed small tubercles on both dorsum and venter and numerous light-brown spots on dorsum (Li *et al.*, 2012a); *R. yaoshanensis* Liu and Hu, 1962 has tangerine on anterior and posterior surface of thighs (Liu and Hu, 1962); *R. yinggelingensis* Chou, Lau and Chan, 2007 has yellow and red-tinged on front of thighs and red on rear of thighs and inner side of shanks (Chou *et al.*, 2007).

Rhacophorus pinglongensis **sp. nov.** is closely related to *R. dorsoviridis*, *R. moltrechti*, and *R. nigropunctatus* based on morphology. Firstly, *Rhacophorus moltrechti* differs from the new species by having large black spots at axillary and lumbar region, flanks of hind limbs bright orange with large black spots, interdigital membrance orange, spotted with black and iris red or reddish brown (Boulenger, 1908). Then, whether *R. nigropunctatus* and *R. dorsoviridis* are conspecific is still contraversial (Orlov *et al.*, 2001; Li *et al.* 2012b; Fei *et al.*, 2012). Herein, we treat them as two valid species, and compare them separately. *R. dorsoviridis* differs from this new species by lacking naptial pads (*vs.* present in the new species), having a single median vocal sac that is not distended (*vs.* exteral single subgular vocal sac); flanks white with variable black spots (*vs.* flanks black with white spots), venter white with a few small black spots below shank (*vs.* venter cream in males); feet and hand yellowish white (*vs.* ventral surface of shanks, dorsal surface of feet, and fingers I, II, III covered with black blotches with white spots or white spots with a faint orange tint); flanks white; but shanks are anteriorly and posteriorly orange (*vs.* black blotches with white spots); the posterior surface and occasionally the dorsomedial surface of tibiotarsus with large black spots (*vs.* large white spots), iris red or yellow above, pale below (*vs.* iris silver, diffusing to ecru laterally with light khaki ring along margin) (Figure 6A-D) (Bourret, 1937; Orlov *et al.*, 2001; Zhang *et al.*, 2011; Fei *et al.*, 2012). Finally, *Rhacophorus nigropunctatus* differs from the new species by having a dark throat; black blotches at flanks and anterior and posterior surfaces of thighs, but lacking white spots; third finger disk width less than tympanum (Liu *et al.*, 1962).

4. Discussion

Preliminary molecular data indicated that *R. pinglongensis* **sp. nov.** and *R. dorsoviridis* (JX219427) are sister species. Although the genetic divergence between *R. pinglongensis* **sp. nov.** and *R. dorsoviridis* (JX219427) is less than 3 % (Table 1), a value usually representing differentiation at the species level in frogs (Vences *et al.*, 2005; Fouquet *et al.*, 2007), morphologically, they can distinguish from each other (Figure 6). Based on Orlov *et al.* (2001) descriptions, *R. dorsoviridis* (JX219427, voucher no. ROM 38011, from Sa Pa, Lao Cai, Vietnam, type locality) has a darken single median vocal sac, obviously differing others *R. dorsoviridis* specimens (males, ROM 38009-38010, 38014-38016, 38018; females, ROM 38006-38008, 38017, from type locality) and this new species. The rest *R. dorsoviridis* specimens (males, ROM 38009-38010, 38014-38016, 38018; females, ROM 38006-38008, 38017, from type locality) are also different from this new species, such as nuptial pad absent and iris brilliant orange above (*vs.* nuptial pad present and iris silver) (Orlov *et al.*, 2001). In addition, we reviewed Zhang *et al.* (2011) descriptions (male, voucher no. KIZ09133-35, from Pingbian, Yunnan, China), those *R. dorsoviridis* specimens obviously differ from *R. pinglongensis* **sp. nov.** by having internal single subgular vocal sac (*vs.* external single subgular vocal sac) (Zhang *et al.* 2011). No matter whether *R. dorsoviridis* came from

Figure 6 (A) The holotype of *Rhacophorus pinglongensis* **sp. nov.**, (B) adult male of *Rhacophorus dorsoviridis* from type locality, Sa Pa, Lao Cai, Vietnam; photo: Nikolai L. Orlov, from Zoological Institute, Russian Academy of Science, (C) adult male of *Rhacophorus dorsoviridis*, dorsolateral view, and (D) ventral view, from Pingbian County, Yunnan, China; photo: Mian Hou, from Sichuan Normal University.

the type locality, Sa Pa, Lao Cai, Vietnam (Figure 6 B; Orlov *et al.*, 2001) or other localities (Figure 6 C, D; Zhang *et al.*, 2011; Fei *et al.*, 2012), *R. pinglongensis* **sp. nov.** can distinguish *R. dorsoviridis* by morphological characters. Ecologically, *R. pinglongensis* **sp. nov.** is known from southern Guangxi at ~500 m elevation and montane evergreen forest, but *R. dorsoviridis* is known from Sa Pa, Lao Cai, Vietnam and Pingbian, Yunnan, China, at 1500–2000 m elevation and evergreen forest (Orlov *et al.*, 2001; Zhang *et al.*, 2011; Fei *et al.*, 2012).

Being consistent with Yu *et al.* (2009) and Li *et al.* (2012b), our results also supported *R. nigropunctatus* is a species complex. Distinct genetic difference indicated *R. nigropunctatus* Longling–Weining group and *R. nigropunctatus* Jinping–Pingbian group are not conspecific. *R. dorsoviridis* (JX219423) is different from *R. dorsoviridis* (JX219427) (Orlov *et al.*, 2001), and has seriously lower interspecies genetic distance (at 0.6 %) with *R. nigropunctatus* Jinping–Pingbian group. *R. nigropunctatus* ranges from Yunnan, Guizhou, Anhui to Hunan provinces, China (Fei *et al.*, 2012). Similar to Li *et al.* (2012b) and Yu *et al.* (2009), our results indicated that *R. nigropunctatus* group contains a cryptic species, where *R. nigropunctatus* from the type locality (Weining, Guizhou) and Pingbian, Yunnan form paraphyletic lineages (Figure 5, sublineage I, II). So we suggest *R. nigropunctatus* Longling–Weining group is *R. nigropunctatus* (Figure 5, sublineage I), which is similar morphologically with the original descriptions of *R. nigropunctatus* (Liu *et al.*, 1962; Li *et al.* 2012b). And *R. nigropunctatus* Jinping–Pingbian group together *R. dorsoviridis* (JX219423) (Figure 5, sublineage II) are *R. dorsoviridis* based on morphology (Bourret, 1937; Orlov *et al.*, 2001). *R. dorsoviridis* (JX219427), as Orlov *et al.* (2001) reported, is distinguished from sublineage I and this news species. Although *R. dorsoviridis* (JX219427) and *R. pinglongensis* **sp. nov.** have lower genetic distance, considering their conspicuous morphology, the taxonomic status of *R. dorsoviridis* (JX219427) need to been investigated to check more material.

So far, females and larval stages are unknown. More breeding ecology of this new species needs to be done to understand them.

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Appendix Comparative mtDNA sequences examined.

ID	Species	Specimen voucher no.	Locality	GenBank No.
1	*Chiromantis xerampelina*	GenBank	Africa	AF458132
2	*Polypedates megacephalus*	GenBank	China	AF458141
3	*P. spinus*	LC0805089	Lvchun, Yunnan, China	JX219416
4	*P. spinus*	LC0805088	Lvchun, Yunnan, China	JX219417
5	*Rhacophorus annamensis*	GenBank	Vietnam	DQ283047
6	*Rhacophorus annamensis*	KIZ64	Bu Gia Map National Park, Bina Phuoc, Vietnam	JX219448
7	*R. arboreus*	GenBank	Japan	AF458142
8	*R. bipunctatus*	CAS229913	Au Yin Ga camp, Nagmung Township,Putao District,Kachin State, Myanmar	JX219445
9	*R. burmanus*	Rao6239	Motuo, Xizang, China	JX219422
10	*R. calcaneus*	GenBank	Tre Don Commune, Tre My, Quang Nam, Vietnam	DQ283380
11	*R. chenfui*	GenBank	Mt. Omei, Sichuan, China	EU215534
12	*R. chenfui*	KIZ060821073	Xichang, Sichuan, China	EF564537
13	*R. chenfui*	Li05	Mt. Omei, Sichuan, China	JX219432
14	*R. chuyangsinensis*	KIZ528	Bi Doup National Park, Lam Dong, Vietnam	JX219450
15	*R. chuyangsinensis*	KIZ746	Bi Doup National Park, Lam Dong, Vietnam	JX219451
16	*R. dennysi*	GenBank	Shaoguan, Guangdong, China	EU215545
17	*R. dennysi*	Li06	Hunan, China	JX219433
18	*R. dennysi*	ZFMK65461	Pet trade	DQ019609
19	*R. dorsoviridis*	ROM38015	Sa Pa, Lao Cai, Vietnam	JX219423
20	*R. dorsoviridis*	ROM38011	Sa Pa, Lao Cai, Vietnam	JX219427
21	*R. duboisi*	ROM38771	Sa Pa, Lao Cai, Vietnam	JX219413
22	*R. duboisi*	ROM38758	Sa Pa, Lao Cai, Vietnam	JX219414
23	*R. dugritei*	SCUM051001L	Baoxing, Sichuan, China	EU215541
24	*R. dugritei*	SCUM051017L	China: Hongya, Sichuan	EU215540
25	*R. dugritei*	KUHE27701	Sichuan, China	LC010584
26	*R. dulitensis*	BORN09097	Sabah, Borneo, Malaysian	AB847123
27	*R. dulitensis*	Rao081201	Malaysia	JX219434
28	*R. feae*	GenBank	Mt. Dawei, Pingbian, Yunnan, China	EU215544
29	*R. feae*	KIZ060821197	Pingbian, Yunnan, China	EF564546
30	*R. gauni*	KUHE53511	Sarawak,Malaysian	AB847124
31	*R. gauni*	FMNH273928	Bintulu Division, Sarawak, Malaysia	JX219456
32	*R. hongchibaensis*	CIB097696	Wuxi, Chongqing, China	JN688882
33	*R. hui*	Li01	Zhaojue, Sichuan, China	JN688878
34	*R. hungfuensis*	GenBank	Wenchuan, Sichuan, China	EU215538
35	*R. kio*	AMS R173451	Nghe An Province,Vietnam	JQ288096
36	*R. kio*	SCUM37941C	Xishuangbanna, Yunnan, China	EU215532
37	*R. malabaricus*	GenBank	India	DQ346957
38	*R. maximus*	Rao6241	Motuo, Xizang, China	JX219411
39	*R. maximus*	GenBank	Vietnam	HM448032
40	*R. minimus*	KIZ061214YP	Mt. Dayao, Guangxi, China	EU215539
41	*R. minimus*	KIZ060821019	Jinxiu, Guangxi, China	EF564560
42	*R. moltrechti*	SCUM061106L	Lianhuachi, Taiwan, China	EU215543
43	*R. moltrechti*	GenBank	Antong, Hualien,eastern Taiwan	DQ468676
44	*R. monticola*	GenBank	Mt. Lompo Batang, Sulawesi Island, Indonesia	AY326060

ID	Species	Specimen voucher no.	Locality	GenBank No.
45	*R. nigropalmatus*	Rao081204	Malaysia	JX219437
46	*R. nigropalmatus*	Rao081203	Malaysia	JX219438
47	*R. nigropunctatus*	Rao060821200	Jinping, Yunnan	JX219424
48	*R. nigropunctatus*	YN080446	Pingbian, Yunnan, China	JX219425
49	*R. nigropunctatus*	Rao060821199	Pingbian, Yunnan, China	JX219426
50	*R. nigropunctatus*	Rao3496	Longling, Yunnan, China	JX219428
51	*R. nigropunctatus*	Rao3494	Longling, Yunnan, China	JX219429
52	*R. nigropunctatus*	GZ070658	Weining, Guizhou, China	JX219430
53	*R. nigropunctatus*	GenBank	Weining, Guizhou, China	EU215533
54	*R. omeimontis*	SC080505	Mt. Omei, Sichuan, China	JX219421
55	*R. omeimontis*	CIB20060104	Sichuan, China	LC010595
56	*R. orlovi*	GenBank	Nga Doi region, Huon Son Reserve, Huong Son District, Ha Tinh, Vietnam	DQ283049
57	*R. pardalis*	FMNH273245	Bintulu Division, Sarawak, Malaysia	JX219453
58	*R. pardalis*	FMNH273243	Bintulu Division, Sarawak, Malaysia	JX219454
59	*R. pingbianensis*	GenBank	Pingbian, Yunnan, China	EU215536
60	*R. pingbianensis*	RaoL060821289	Jinping, Yunnan, China	JX219415
61	*R. puerensis*	GenBank	Puer, Yunnan, China	EU215542
62	*R. puerensis*	ROM37996	Lao Cai, Sa Pa, Vietnam	JN688891
63	*R. reinwardtii*	Rao081205	Malaysia	JX219443
64	*R. rhodopus*	GenBank	Hainan, China	EU215529
65	*R. rhodopus*	ROM 99944	Vietnam	AF458144
66	*R. rhodopus*	Lc0805109	Lvchun, Yunnan, China	JX219440
67	*R. rufipes*	FMNH272858	Bintulu Division, Sarawak, Malaysia	JX219455
68	*R. schlegelii*	GenBank	Japan	AB202078
69	*R. schlegelii*	GenBank	Hiroshima City, Western Japan	NC007178
70	*R. sp.*	03309Rao	Maguan, Wenshan, Yunnan, China	JX219435
71	*R. sp.*	Rao03324	Malipo, Wenshan, Yunnan, China	JX219408
72	*R. sp.*	Rao03326	Malipo, Wenshan, Yunnan, China	JX219409
73	*R. sp.*	Rao03321	Malipo, Wenshan, Yunnan, China	JX219410
74	*R. sp.*	FMNH235741	Kota Marudu District, Sabah, Malaysia	JX219452
75	*R. taronensis*	SCUM 060614L	Mt. Gaoligong, Yunnan,China	EU215537
76	*R. translineatus*	Rao6237	Motuo, Xizang, China	JX219449
77	*R. verrucopus*	6254Rao	Motuo, Xizang, China	JX219436
78	*Spinomantis peraccae*	GenBank	Africa	DQ283036
79	***R. pinglongensis* sp. nov.**	**NHMG201002003**	**Mt. Pinglong, Shangsi, Guangxi, China**	**KU170683**
80	***R. pinglongensis* sp. nov.**	**NHMG201002011**	**Mt. Pinglong, Shangsi, Guangxi, China**	**KU170684**

ROM, Royal Ontario Museum, Toronto, Canada; FMNH, Field Museum of Natural History, Chicago, USA; CAS, California Academy of Sciences, San Francisco, USA; KIZ, Kunming Institute of Zoology, the Chinese Academy of Sciences, Kunming, China; ZFMK, Zoologisches Forschungsinstitut und Museum A. Koenig, Bonn, Germany; NHMG, Natural History Museum of Guangxi, Nanning, China; CIB, Chengdu Institute of Biology, the Chinese Academy of Sciences, Chengdu, China; SCUM, Sichuan University Museum, Chengdu, China.